REMARKS

Prior to entry of this paper, claims 31-41 and 46-62 were pending in this application with claims 46 and 47 being withdrawn. In this paper, Applicants have amended claims 31-33, 36, 37, 39, and 51-53, have canceled claims 34, 35, 48, 49, 57-59, and 62, and have introduced new claims 63-65. After entry of this paper, claims 31-33, 36-41, 50-56, 60, 61, and 63-65 will remain pending and are presented for consideration. Applicants submit that the amendments add no new matter.

Interview Summary

Applicants would like to thank Examiner Jung for her time and helpful suggestions during the in person interview conducted at the U.S. Patent and Trademark Office on May 4, 2009. During the interview, Applicants discussed with the Examiner the Hoylaerts, Handin, Favaloro, and Vischer references and explained how there is no motivation to combine these references to obviate Applicants' claimed invention to distinguish between type 2A and type 2B von Willebrand's disease (vWD). The Examiner agreed that such a motivation did not exist because Hoylaert's assay is not sensitive enough to discriminate between these two types of vWD.

Applicants also discussed with the Examiner that Applicant's method utilizes an antibody to present the soluble form or portion of GP1b(α). The Examiner suggested amending the claim to recite that GP1b(α) is presented by an antibody and to provide a declaration relating to the feature of the antibody-presented GP1b(α).

Applicants also discussed with the Examiner how Applicants' claimed method meets the long-felt need in the art for an improved assay for discriminating among types of von-Willebrand disease.

Support for Claim Amendments

Claim 31 has been amended to recite a method for discriminating between types of von-Willebrand disease using a von-Willebrand factor binding activity wherein the soluble form or portion of GP1b(α) is presented by an anti-GP1b(α) antibody. Support for these amendments are found in the application as originally filed at page 5, lines 20-22; at page 8, lines 7-9; at page 9,

lines 22-30; at page 11, lines 25-27; and at page 27, line 23 to page 28, line 16, with page and line numbers referring to the international application publication WO 01/02853.

Claim 32 has been amended to recite a soluble form or portion of GP1b(α). Support for this amendment is found in the application as originally filed at page 5, lines 23-24 of the published international application.

Claim 33 has been amended to recite that the anti-GP1b(α) antibody is bound to a solid support. Support for this amendment is found in the application as originally filed at page 8, lines 7-9 of the published international application.

Claims 36, 37, 39, and 51-53 have been amended for antecedent basis purposes in view of amendments to claims from which they depend.

Support for new claim 63 is found in the application as originally filed at page 26, lines 25-27 of the published international application.

Support for new claim 64 is found in the application as originally filed at page 11, lines 18-19 of the published international application.

Support for new claim 65 is found in the application as originally filed at page 11, lines 25-27.

Brief Discussion of Applicants' Claimed Invention

Applicants' claimed invention is directed to a method for discriminating between types of von-Willebrand disease (vWD) using a von-Willebrand factor (vWF) binding activity. The method comprises the steps of detecting the binding activity of von-Willebrand factor (vWF) in a sample, determining an amount of vWF-antigen in the sample, determining a ratio between the binding activity and the amount of vWF-antigen, comparing the ratio to a reference range, and discriminating between types of vWD using the comparison.

According to Applicants' claimed invention, the binding activity detected is the binding activity of vWF in a sample to a soluble form or a portion of GP1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. The soluble form or a portion of GP1b(α) that is not associated with a platelet is presented by an anti-GP1b(α) antibody. This binding activity is a measure of the ristocetin-dependent (or functionally

equivalent substance of ristocetin) binding between GP1b(α) (or a portion thereof) and vWF. This is also known as the *ristocetin cofactor activity*.

Prior to Applicants' claimed invention, no one had successfully used a soluble form or portion of GP1b(α), such as glycocalicin or any other soluble fragment of GP1b(α), that is not associated with a platelet, to detect the ristocetin cofactor activity, thereby discriminating between different types of vWD. Rather, prior art vWD assays used whole platelets from a normal subject. Applicants discovered for the first time that a soluble form or portion of GP1b(α), when presented by an anti-GP1b(α) antibody can be used successfully to obtain the vWF ristocetin cofactor activity with a sensitivity requisite for discriminating between types of vWD and useful for diagnosing patients suspected to have vWD.

Applicants' claimed invention provides a significantly improved ristocetin cofactor activity assay over for discriminating between types of vWD those known in the art. The improvements provided by Applicants' assay meet a long felt need in the art that has persisted over approximately the last 35 years for an assay that can detect extremely low levels of vWF present in certain types of vWD patients and thereby provide a more accurate diagnosis of the type of vWD a patient has.

In particular, Applicants' claimed method has reduced intra and interassay variability and improved sensitivity to detect very low levels of vWF as compared to other prior art assays. Accordingly, it meets this long felt need.

Based on the arguments presented in the responses filed on February 22, 2007, November 20, 2007, and August 20, 2008, which are incorporated by reference and for the reasons set forth below, Applicants respectfully submit that the claimed invention is not obvious and therefore is patentable.

I. Rejection of Claims 31-41, 48-53, 56-60 and 62 Under 35 U.S.C. § 103(a) in view of Favaloro, Vischer, Hoylaerts, and Handin

Claims 31-41, 48-53, 56-60 and 62 stand rejected under 35 U.S.C. § 103(a) over Favaloro *et al.*, (Pathology, 25:152-158 (1993) ("Favaloro")) in view of Vischer *et al.* (Critical Reviews in Oncology/Hematology, 30:93-109 (1999) ("Vischer")), in view of Hoylaerts *et al.* (Biochem. J. 386:453-463 (1995) ("Hoylaerts")), and in view of Handin (U.S. Patent No. 5,321,127

("Handin")). Applicants have canceled claims 34, 35, 48, 49, 57, 58, 59, and 62. Accordingly, Applicants respectfully request that the rejection of these claims be withdrawn. Applicants traverse the remaining rejections to the extent they are maintained of the claims as amended.

1. Summary of the Rejection

At page 4-5, the Office action suggests that Favaloro teaches a method for detecting vWD according to the steps of Applicants' claimed invention set forth in claim 31 except that Favaloro teaches a ***collagen binding assay*** which detects vWF binding to collagen rather than the claimed step of detecting a binding activity of vWF in a sample to a soluble form or a portion of glycoprotein 1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance (Office action, pg. 4, lines 11-12; pg. 5, lines 14-16).

The Office action asserts that this deficiency of Favaloro is found in the teachings of Hoylaerts and Handin.

Hoylaerts teaches how ristocetin mediates binding of vWF to GP1b purified from platelets. Hoylaerts teaches that specific vWF binding to the natural GP1b receptor is obtained at restricted concentrations of ristocetin (i.e., 0.4 mg/mL). (See page 454, LH col., Materials and Methods; page 462, LH col., discussion, 3rd full para.).

Handin teaches a soluble recombinant GP1b(α) fragment polypeptide rGP1b(α)(Q_{221}-L_{318}) that inhibits ristocetin-dependent binding of vWF to platelets (col. 3, lines 16-18). Handin also teaches that purified glycocalicin (GC), which contains the majority of the extracellular portion of the GP1b(α) chain can inhibit ristocetin-dependent binding of vWF to platelets (col. 2, lines 51-61).

In view of these teachings, the Office action asserts that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to employ "the vWF binding activity detection method of Hoylaerts" using the GP1b(α) of Handin, in addition to the collagen binding assay of Favaloro in order to arrive at Applicants' claimed invention. The Office action suggests that a skilled artisan would be motivated to do so in order to differentiate between type 2A vWD and type 2B vWD (Office action, page 9, lines 19-page 10, line 2; page 8, lines 17-20). The Office action asserts that there would be further motivation to do so and a reasonable expectation of success because the assay of Hoylaerts has the alleged advantage of

detecting vWF activity without the contributions of non-specific ristocetin-dependent molecular interactions when isolated GP1b is used. Further, the Office action suggests there would be motivation to use the GP1b(α) fragment of Handin which contains the ristocetin-dependent vWF binding site in place of Hoylaerts isolated GP1b which contains components that are not involved in specific vWF binding (page 10, line 19 to page 11, line 6).

In contrast to the Office action's assertions, Applicants respectfully submit that none of Vischer, Hoylaerts, or Handin cures the deficiencies of Favaloro, nor would a skilled artisan have the motivation or reasonable expectation of success at combining the references as suggested by the Office action for the reasons outlined below.

2. It is not obvious to detect vWF binding to GP1b(α) presented by an anti-GP1b(α) antibody in a method for differentiating between types of vWD.

A. None of the references cited by the Office action teach or suggest detecting vWF binding to GP1b(α) presented by an anti-GP1b(α) antibody, a required element of the claimed invention, in a method for differentiating between types of vWD.

Applicants have amended claim 31 to recite the step of detecting the binding activity of vWF in a sample to a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. The soluble form or portion of GP1b(α) is presented by an anti-GP1b(α) antibody.

Applicants submit that none of Favaloro, Vischer, Hoylaerts or Handin teaches or suggests, either alone or in combination, detecting vWF binding activity to soluble GP1b(α) or a portion thereof in the presence of ristocetin by antibody presentation of GP1b(α) with an anti-GP1b(α) antibody. Accordingly, this combination of references fails to teach all the required steps of Applicants' claimed invention and therefore cannot render Applicants' invention obvious.

Favaloro teaches detecting vWF binding activity to collagen (Favaloro, pg. 153; Office action page 4, lines 9-13). Favaloro does not teach detecting vWF binding activity to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in the presence of ristocetin. In fact, Favaloro does not teach GP1b(α) or an anti-GP1b(α) antibody, let alone presentation of

GP1b(α) by an anti-GP1b(α) antibody. Accordingly, Applicants submit that it would not be obvious, based on the teachings or Favaloro, to present GP1b(α) via an anti-GP1b(α) antibody in order to detect vWF binding activity to GP1b(α) in the presence of ristocetin in order to discriminate between different types of vWD.

Applicants submit that the teachings of Vischer do not cure the deficiencies of Favaloro. Vischer, like Favaloro, also does not teach detecting vWF binding activity to soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in the presence of ristocetin. In fact, Vischer does not even teach a soluble form or portion of GP1b(α) or an anti-GP1b(α) antibody, let alone presentation of GP1b(α) by an anti-GP1b(α) antibody. Accordingly, Applicants submit that it would not be obvious, based on the teachings of Vischer, alone, or in combination with the teachings of Favaloro, to present GP1b(α) via an anti-GP1b(α) antibody in order to detect vWF binding activity to GP1b(α) in the presence of ristocetin in order to discriminate between different types of vWD.

Applicants submit that the teachings of Hoylaerts do not cure the deficiencies of Favaloro and Vischer. Hoylaerts, like Favaloro and Vischer, does not teach detecting vWF binding activity to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in the presence of ristocetin. While Hoylaerts teaches assaying vWF binding to GP1b isolated from platelets in the presence of ristocetin, Hoylaerts does not teach antibody presentation of GP1b. Hoylaerts presents GP1b by coating the wells of microtiter plates with purified GP1b, (pg. 454, LH col., 4th para.), not by using an antibody. Further, the antibodies taught by Hoylaerts are anti-vWF antibodies and a non-specific control antibody, none of which are an anti-GP1b(α) antibody. Moreover, none of Hoylaert's antibodies is used to present a soluble form or portion of GP1b(α). For all these reasons, a skilled artisan, based on the teachings of Hoylaerts alone, or in combination with the teachings of one or more of Favaloro and Vischer, would not be motivated to present GP1b(α) via an anti-GP1b(α) antibody in order to detect vWF binding activity to GP1b(α) in the presence of ristocetin in order to discriminate between different types of vWD.

Applicants submit that the teachings of Handin fail to remedy the deficiencies of Favaloro, Vischer, and Hoylaerts. Handin, like Favaloro, Vischer, and Hoylaerts fails to teach

detecting vWF binding activity to soluble form or portion of GP1b(α) presented by an anti-GP1b(α) in the presence of ristocetin. While, Handin teaches a GP1b(α) fragment (col. 3, line 15-16), Handin's GP1b(α) fragment is not presented via an antibody for binding to vWF. Further, while Handin teaches an anti-GP1b(α) antibody, Handin's anti-GP1b(α) antibody is used merely for purifying a GP1b(α) fragment in an ELISA (col. 16, lines 9-11). Accordingly, based on the teachings of Handin alone, or in combination with the teachings of one or more of Favaloro, Vischer, and Hoylaerts, a skilled artisan would not be motivated to present GP1b(α) via an anti-GP1b(α) antibody in order to detect vWF binding activity to GP1b(α) in the presence of ristocetin in order to discriminate between different types of vWD.

Given that none of Favaloro, Vischer, Hoylaerts, or Handin, either alone or in combination teaches or suggests presenting a soluble form or portion of GP1b(α) by an anti-GP1b(α) antibody, let alone in a method for detecting vWF binding activity to GP1b(α) in the presence of ristocetin, Applicants submit that Applicants claimed invention is not obvious.

B. The Second Declaration of Dr. Deckmyn provides further evidence that detecting vWF binding to GP1b(α) presented by an anti-GP1b(α) antibody in a method for differentiating between types of vWD was not obvious to a skilled artisan at the time of Applicants' invention.

Applicants submit that they were the first to recognize the utility of GP1b(α) isolated from a platelet, as opposed to using the whole platelet, in an assay to detect the ristocetin cofactor activity of vWF, *i.e.*, the binding of vWF to GP1b(α) or a portion thereof in the presence of ristocetin or a functionally equivalent substance (Second Declaration of Dr. Hans Deckmyn ("Deckmyn II") at para. 12, filed herewith).

As detailed in the Second Declaration of Dr. Deckmyn, Applicants discovered that GP1b(α) cannot bind vWF in the presence of ristocetin when GP1b(α) is merely immobilized on a surface, such as plastic (see Deckmyn II, para. 13). As discovered by Applicants, when the naturally occurring soluble form of GP1b(α), glycocalicin, was immobilized on plastic well plates, the binding activity of vWF to glycocalicin in the presence of ristocetin was negligible (Deckmyn II, para. 13). When the GP1b(α) fragment, $GP1b(\alpha)_{(1\text{-}289)}$, was immobilized on plastic well plates, the binding activity of vWF to $GP1b(\alpha)_{(1\text{-}289)}$ in the presence of ristocetin was also

negligible (Deckmyn II, para. 13). Such negligible binding activities indicated that immobilized GP1b(α) was not useful for a clinical assay for detecting vWF binding to GP1b(α) (Deckmyn II, para. 13). Applicants determined that vWF was unable to bind the immobilized GP1b(α) fragments because immobilization caused a conformation change in GP1b(α) which prevented vWF binding to GP1b(α) (Deckmyn II, para. 13). For these reasons, Applicants determined that assaying vWF binding activity to a soluble form or portion GP1b(α) immobilized to a solid support in the presence of ristocetin would not be an effective substitute for detecting vWF ristocetin cofactor activity in the presence of platelet-GP1b because the solid support-immobilized GP1b(α) fragments did not permit detection of any significant vWF binding activity (Deckmyn II, para. 13).

Given the difficulties in detecting vWF binding activity to immobilized GP1b(α), Applicants submit that it is hardly obvious that vWF binding activity to GP1b(α) could be detected when portions of GP1b(α) were presented by an anti-GP1b(α) antibody. Nevertheless, Applicants discovered that in order to detect the binding activity of vWF to GP1b(α) or a fragment thereof, it was necessary to present the soluble form or portion of GP1b(α) by an anti-GP1b(α) antibody (Deckmyn II, para. 14). Applicants screened over 90 monoclonal antibodies to GP1b(α) to determine if the $GP1b(\alpha)_{(1\text{-}289)}$ fragment, when bound to any of these antibodies, would bind vWF (Deckmyn II, para. 14). Sufficient levels of vWF binding to the $GP1b(\alpha)_{(1\text{-}289)}$ fragment in the presence of ristocetin were detected for three anti-GP1b(α) antibodies. Applicants also screened over 40 monoclonal anti-GP1b(α) antibodies to determine if glycocalicin, when bound to any of these antibodies, would bind vWF. Sufficient levels of vWF binding to glycocalicin in the presence of ristocetin were detected for one anti-GP1b(α) antibody. Accordingly, from this it was determined that these anti-GP1b(α) antibodies would be suitable for presenting a soluble form or portion of GP1b(α) in an assay for detecting vWF binding to GP1b(α) or a fragment thereof (Deckmyn II, para. 14).

Further, as demonstrated in Applicants' specification, Applicants were the first to determine that GP1b(α) presentation via an anti-GP1b(α) antibody was sufficiently robust to detect not only normal vWF binding activity to GP1b(α) but also defective vWF. Applicants specification describes how vWF from Type 1, Type 2A, Type 2B, Type 2N, and Type 3 vWD

patients was assayed for vWF binding activity to GP1b(α) presented via an anti-GP1b(α) antibody (see page 22, lines 7-23 and at page 27, line 11 to page 28, line 16 of international publication). As taught in the specification, the values for vWF activity detected via antibody presentation of GP1b(α) correlated with known values of vWF for the vWD patient samples detected using whole platelets, indicating that GP1b(α) presented via an anti-GP1b(α) antibody is sufficiently robust to detect vWF binding activity in diseased patients (see page 28, lines 14-16).

Given the challenges met by Applicants in successfully detecting vWF binding activity to a soluble form or portion of GP1b(α), Applicants submit that it was not obvious at the time of Applicants' invention to use GP1b(α) as a substitute for platelet-GP1b in a clinical assay for detecting the ristocetin cofactor activity of vWF, let alone to present the GP1b(α) via an anti-GP1b(α) antibody in order to detect the vWF binding to GP1b(α) in the presence of ristocetin.

C. Conclusion

For all the reasons set forth above in Sections 2A and 2B, Applicants submit that the claimed invention as amended which requires detecting vWF binding activity to a soluble form or portion of GP1b(α) absent a platelet in the presence of ristocetin wherein GP1b(α) is presented by an anti-GP1b(α) antibody in a method for discriminating between types of vWD is not obvious. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

3. Applicants' invention is not obvious because there is no motivation to combine the references.

In addition to the fact that the combination of references—Favaloro, Vischer, Hoylaerts, and Handin—cited by the Office action fails to teach all of the elements of Applicants' claimed invention, Applicants submit that there is no motivation to combine the references to render Applicants' claimed invention obvious for the following reasons.

A. GP1b is a part of a complex protein that cannot be dissembled with retained functionality of the GP1b portion.

Applicants submit that a skilled artisan would not be motivated to use only GP1b(α) or a portion thereof in the absence of a platelet in order to detect the vWF binding to GP1b(α) in the presence of ristocetin. GP1b(α) is one of many polypeptides comprising the glycoprotein 1b-IX complex. The GP1b-IX complex includes two GP1b(α) subunits, two GP1bβ subunits, two GPIX subunits, and one GPV subunit (Lopez, (1994), "The platelet glycoprotein 1b-IX complex, Blood Coag. Fibrin., 5:97-119 at 97-98, attached as "Exhibit A"). Applicants submit that a skilled artisan would not be motivated to use GP1b(α) or a portion thereof isolated from the complex of GP1b-IX because there is no certainty that GP1b(α) or a portion thereof, absent the entire complex, would be sufficiently sensitive to detect binding with normal vWF and binding with defective vWF.

In order to discriminate between different types of vWD, it is necessary that an *in vitro* assay mimic the *in vivo* activity of vWF. Applicants submit that a skilled artisan would have no expectation that normal and defective vWF would interact with isolated GP1b(α) in the same fashion as they would with the GP1b-IX complex. A skilled artisan would expect that any assistance provided to vWF by other portions of the complex to assist in binding would be lost because the conformation of isolated GP1b(α) may be different from its native conformation in the GP1b-IX complex, affecting the binding of vWF. Accordingly, any binding detected between vWF to isolated GP1b(α) would not be expected by the skilled artisan to mimic the *in vivo* binding activity of the vWF assayed. An inability to detect the actual binding activity of vWF would result in errors in discriminating between different types of vWD based on the results of the assay. Moreover, as discussed above in Section 2B, Applicants found that isolated GP1b(α) did not bind sufficiently to vWF *in vitro* without modifying GP1b(α) presentation.

For this reason alone, Applicants submit that a skilled artisan would not be motivated to present GP1b(α) or a portion thereof with an anti-GP1b(α) antibody to detect the binding activity of vWF to GP1b(α) in the presence of ristocetin. Accordingly, Applicants submit that the claimed invention is not obvious in view of the references cited in the Office action. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-

41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

B. There is no motivation or reasonable expectation of success in combining the references to differentiate between Type 2A and Type 2B vWD.

The Office action's motivation and reasonable expectation of success to combine the teachings of Favaloro, Hoylaerts, and Handin, as suggested above, in order to differentiate between type 2A and type 2B vWD rests on teachings of Vischer (Office action page 9, line 19 to page 10, line 1). Vischer teaches that Type 2A vWD is characterized by qualitative variants of VWF with decreased platelet-dependent function that is associated with the absence of high molecular weight multimers of vWF and that Type 2B vWD is characterized by qualitative variants of vWF with increased affinity for platelet GP1b and by increased platelet agglutination at low concentrations of ristocetin (Table 2, page 99; page 100, LH col., 6.2.2.).

The Office action suggests that a skilled artisan would have had a reasonable expectation of success in creating the claimed method because Vischer teaches that "a variety of screening assays can be employed to further define vWD" (Office action, page 10, lines 11-15; Vischer p. 102). Applicants respectfully disagree for the reasons provided below

i. Hoylaerts does not disclose detecting vWD.

Firstly, Applicants submit that a skilled artisan would not be motivated to use the teachings of Hoylaerts to create an assay for detecting vWF binding activity to GP1b in the presence of ristocetin, let alone to differentiate between Type 2A and Type 2B vWD using such an assay because Hoylaerts does not teach a "vWF binding activity detection method" as asserted by the Office action (page 9, lines 20-21).

In particular, Applicants submit that Hoylaerts does not teach a method for discriminating between types of vWD that involves detecting vWF activity in a sample, let alone a method for detecting vWF activity in a sample using a soluble form or a portion of glycoprotein 1b (GP1b) and ristocetin as suggested by the Office action (page 6, lines 8-10).

Hoylaerts teaches methods for assessing the specificity of ristocetin-dependent interactions between GP1b and vWF at varying concentrations of ristocetin (p. 457, RH col.).

Hoylaerts' vWF is isolated from plasma samples using an ELISA method (p. 454, LH col.). Accordingly, Hoylaerts is not assaying patient samples but is assaying predetermined concentrations of vWF to study binding of GP1b to vWF. Hoylaerts provides no suggestion that the concentrations of vWF tested in Hoylaert's examples are relevant to levels of normal or defective vWF found in normal or vWD patients. Accordingly, there is no teaching in Hoylaerts that GP1b in the absence of a platelet is sensitive enough in an assay to detect vWF binding in normal or vWD patients, which binding may be extremely low depending on concentrations of vWF present in a the particular type of vWD.

Further, Hoylaerts does not teach or suggest that an isolated GP1b fragment can be useful in differentiating between different types of vWD. In fact, *Hoylaerts does not assay plasma samples from any vWD patients to determine if vWF from diseased patients interacts with GP1b in the presence of ristocetin in the same fashion as when GP1b is associated with a platelet.* Rather, *Hoylaerts tested only normal vWF* purified from a human plasma sample (p. 454, LH col.). Accordingly, Hoylaerts is silent as to whether Hoylaerts' method for assessing the ristocetin-mediated binding of *normal* vWF to GP1b is sufficiently sensitive or robust to detect binding activities between *defective* vWF and GP1b. Accordingly, there is no indication in Hoylaerts' that using GP1b *in the absence of platelets* would provide clinically relevant data for discriminating between types of vWD.

Applicants submit that for these reasons, Hoylaerts does not cure the deficiencies of Vischer and Favaloro as all of these references fail to teach using GP1b, let alone GP1b(α) presented by an anti-GP1b(α) antibody to detect binding activity with vWF in the presence of ristocetin in a method for discriminating between different types of vWD.

Further, Given that Type 2A and Type 2B vWD are each characterized by defective variants of vWF (Deckmyn II, paras. 5 and 6), Applicants fail to see any reason why a skilled artisan would be motivated to use Hoylaert's teachings regarding ristocetin-mediated binding between GP1b and **normal** vWF in order detect vWF activity in a patient sample suspected of having **defective** vWF, *i.e.*, a sample from a patient having a type 2A or type 2B vWD.

ii. Christophe teaches away from using GP1b to detect defective vWF binding activity.

That GP1b or a fragment thereof is not sensitive or robust enough to detect binding activities between *defective* vWF and GP1b is supported by the teachings of Christophe ((1994),Blood, 83(12):3553-3561), cited in the October 23, 2006, Office action. Christophe compares the binding capacity of *defective* vWF from type 2A vWF, type 2B vWF, and normal vWF to a soluble fragment of GP1b(α), glycocalicin (pg. 3554, LH col., lines 1-4). Christophe found that the binding of plasma vWF from type 2A vWD patients, type 2B vWD patients, and normal patients to glycocalicin in the presence of saturating concentrations of botrocetin, a functional equivalent of ristocetin, "was not significantly different" (Christophe, pg. 3560, LH col. 2nd paragraph, abstract 2nd col.). In other words, Christophe disclosed that the binding activity detected between normal and defective vWF in a plasma sample and a soluble fragment of GP1b(α), glycocalicin, did not provide clinically relevant data to allow discrimination between normal samples and samples from patients with vWD.

Accordingly, based on the teachings of Christophe who assayed *defective* vWF binding to the GP1b(α) fragment glycocalicin, a skilled artisan would be taught away from using GP1b(α) or a fragment thereof absent a platelet because a skilled artisan would believe that the us of that GP1b(α) or a fragment thereof is not sensitive enough to distinguish between normal and defective vWF. A skilled artisan would therefore conclude that a GP1b(α) or a fragment thereof would not be suitable in an assay for detecting vWF binding in order to discriminate between different types of vWD.

For all the reasons set forth in Sections 3B(i) and (ii) above, Applicants' submit that a skilled artisan would not conclude that the method of Hoylaerts would be useful to differentiate between type 2A vWD and type 2vWD as suggested by the Office action when the art provides no teaching that GP1b(α) or a fragment thereof would be suitable in an assay for discriminating between different types of vWD.

iii. The absence of high molecular weight multimers of vWF in Type 2A vWD is not a basis for discriminating between Type 2A and Type 2B vWD.

The Office action suggests that a skilled artisan would have been motivated to combine the teachings of the references to arrive at Applicants claimed invention. The motivation allegedly lies in the skilled artisan's desire to distinguish between Type 2A vWD and Type 2B vWD (Office action, page 9, line 19-page 10, line4). The Office action suggests this could have been done given that Type 2A vWD is characterized by a lack high molecular weight multimers of vWF. (page 9, line 19 to page 10, lines 3). Applicants submit, however, that while type 2A vWD is characterized by the absence of high molecular weight multimers of vWF, type 2B vWD is also characterized by the absence of high molecular weight vWF multimers (Deckmyn II, para. 5) for the reasons explained below. Therefore, Type 2A and Type 2B vWD cannot be differentiated from one another by detecting an absence of high molecular weight vWF multimers (HMW vWF) as the plasma of both Type 2A and Type 2B vWD patients is deficient in HMW vWF.

iv. Type 2B vWD's increased affinity for platelet GP1b is not a basis for discriminating between Type 2A and Type 2B vWD.

While Type 2B is characterized by variant vWF with increased affinity for platelet GP1b, as suggested by the Office action (page 10, lines 4-5), Applicants submit that a skilled artisan, at the time of the invention, would have realized that differentiation between type 2A and type 2B would not be possible based on this characteristic for the following reasons.

Type 2B vWD is characterized by increased affinity of vWF for platelet GP1b because of a genetic defect in Type 2B vWD patients such that Type 2B vWF has a higher than usual affinity for GP1b (Deckmyn II, para. 6). As a result, Type 2B vWF readily binds to platelet GP1b in circulating blood and is rapidly cleared from the blood (Deckmyn II, para. 6). This accounts for the absence of free (unbound to platelet GP1b) HMW vWF in type 2B patients (Deckmyn II, para. 6). Accordingly, if blood, plasma, or serum from a Type 2B patient sample were incubated with ristocetin and isolated GP1b, a skilled artisan would expect to detect only low amounts of vWF binding, including binding of HMW vWF, to isolated GP1b because the HMW vWF Type 2B vWF would already be bound to the patient's own platelets and/or removed

from circulation and therefore be unavailable for any significant level of binding to the free GP1b (Deckmyn II, para. 7). Accordingly, minimal binding from vWF monomers to GP1b would contribute to the low amount of vWF detected.

The same result would also be obtained for a Type 2A patient. While type 2A is not characterized by increased affinity for platelet GP1b, Type 2A patients also present with an absence of HMW vWF (Deckmyn II, para. 5). The absence of high molecular weight vWF is attributable to errors in protein synthesis that produce only small fragments or monomers that cannot multimerize and therefore have a decreased capacity to interact with platelet GP1b (Deckmyn II, para. 5). Because HMW vWF are generally responsible for binding to GP1b, if blood, plasma, or serum from a Type 2A patient were incubated with ristocetin and GP1b, a skilled artisan would expect to detect only low binding of HWM vWF to GP1b because of the absence of HMW vWF in the patient sample and the reduced capacity of remaining smaller vWF multimers to bind GP1b (Deckmyn II, paras. 5 and 8). Accordingly, whether a skilled artisan were assaying a Type 2A or a Type 2B patient sample, the predicted vWF binding in the presence of ristocetin and GP1b would be the same low level for both Type 2A and Type 2B (Deckmyn II, paras. 7-9). Accordingly, it would not be possible to distinguish between Type 2A and Type 2B vWD based on such data.

v. Hoylaerts' method could not be used to distinguish between Type 2A and Type 2B vWD based on Type 2B vWD's characteristic of increased platelet agglutination at low concentrations of ristocetin.

While the Office action points to the teachings of Vischer as teaching that type 2B vWD is characterized by increased platelet agglutination at low concentrations of ristocetin (Office action, page 6, lines 5-7), Applicants submit that it is not possible to differentiate between Type 2A and Type 2B vWD by detecting increased platelet agglutination at low concentrations of ristocetin using the isolated GP1b and ristocetin of Hoylaerts for the following reasons.

Firstly, as taught by Vischer, (pg. 103, LH col., paragraph 7.2.2.1.; Table 4), type 2B vWD is characterized by increased agglutination of the patient's own platelets at low concentrations of ristocetin, as opposed to exogenously introduced platelets for the reasons provided below (see also Deckmyn II at para. 10).

The assay described by Vischer for testing the agglutination of the patient's own platelets is called a ristocetin induced platelet agglutination assay (RIPA) (Vischer, pg. 103). Because Type 2B vWD vWF has a high affinity for platelet-GP1b, most of a Type 2B patient's vWF is already bound to platelet-GP1b or closely associated with it (see Deckmyn II, para. 6 and 10). Accordingly, a Type 2B patient's own platelets will aggregate in response to low concentrations of ristocetin because Type 2B defective vWF does not require the assistance from ristocetin required by normal vWF to bind GP1b on a normal patient's own platelets (Deckmyn II, para. 10). In contrast, Type 2A vWD patient platelets exhibits little to no aggregation in the presence of the patient's own platelets and ristocetin because, as previously explained, Type 2A vWF contains only small vWF multimers which have a low affinity for GP1b, binding to which is necessary to cause platelet aggregation (Deckmyn II, paras. 5 and 10).

Further, even if the isolated GP1b of Hoylaerts were used in addition to platelets from a patient having Type 2B vWD, as required by Vischer's RIPA assay, the isolated GP1b would compete with platelet GP1b from the patient for binding to defective Type 2B vWF, interfering with the results (Deckmyn II, para. 11). In other words, some Type 2B vWF binds to the patient platelets and some to isolated GP1b, reducing agglutination of patient platelets. Further, reduced levels of agglutination would also be seen with patient platelets from a Type 2A vWD patient. Because of the reduced levels of agglutination from the expected levels needed to distinguish between Type 2A and Type 2B vWD, it would not be possible to accurately distinguish between Type 2B vWD and Type 2A vWD based on this method (Deckmyn II, para. 11). Accordingly, the position of the Office action is based on a misunderstanding of the science underlying the invention. While it is true that Type 2B vWD can be distinguished from Type 2A by increased agglutination of a Type 2B patient's own platelets at low concentrations of ristocetin, if this were tested in the presence of Hoylaerts' isolated GP1b, it would not be possible to accurately distinguish Type 2B vWD from Type 2A vWD (Deckmyn II, para. 11).

Further, Applicants submit that the method of Hoylaerts could not be used to differentiate Type-2B from Type-2A vWD as asserted by the Office action because it does not have all the necessary components required to perform a RIPA assay, *i.e.*, Hoylaerts does not use the patient's own platelets from the patient's plasma sample being tested. In fact, Hoylaerts does not

use any platelets. For this further reason, Applicants submit that one skilled in the art would not look to the teachings of Favaloro and Vischer and combine them with Hoylaerts' method in order to differentiate subtype 2A and 2B vWD because Hoylaert's does not teach a RIPA assay, the assay necessary according to Vischer to distinguish type 2B from other subtypes of type 2 vWD (see pg. 103, LH col., paragraph 7.2.2.1.; Table 4).

Applicants further submit that even if Hoylaert's taught a RIPA assay, which he does not, such a teaching is irrelevant to Applicants' claimed invention as Applicants' claimed method does not require performing a RIPA assay. Rather, the binding activity detected in Applicants' claimed invention is the ristocetin cofactor activity, *i.e.*, the binding activity of vWF in a sample to a soluble form of GP1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. Because the claimed GP1b(α) fragment is not associated with the patient's own platelets, the claimed step cannot be a RIPA assay (Declaration of Dr. Bruguera submitted August 20, 2008, paras. 4-5, "Bruguera I"). Rather, vWF activity determined from agglutination in the presence of ristocetin and exogenous platelets (from a source other than the patient) or a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet is the *ristocetin cofactor activity* (see Bruguera I, paras. 4-5).

Further, Applicants submit that even if Hoylaerts method could detect ristocetin cofactor activity according to Applicants' claimed invention, which Applicants' submit it is not, an assay for ristocetin cofactor activity cannot differentiate between type 2A and type 2B vWD because, as stated in the first Declaration of Dr. Hans Deckmyn (filed February 22, 2007, "Deckmyn I") at paragraph 7, "the ristocetin cofactor assay [is] capable of detecting patients with subtypes 2A and 2B von Willebrand's disease." Accordingly, Applicants submit that there would be no motivation to combine the teachings of Favaloro, Vischer, and Hoylaerts based on the Office action's rationale of differentiating type 2A and 2B vWD because such a differentiation cannot be made with a ristocetin cofactor activity assay.

vi. Favaloro's collagen binding assay alone, or in combination with Hoylaert's "assay" cannot differentiate between Type 2A and Type 2B von Willebrand's disease.

The collagen binding assay of Favaloro which the Office action combines with the teachings of Vischer and Hoylaerts likewise does not permit differentiation between type 2A and

type 2B vWD because, as stated by Dr. Deckmyn, "the CBA [(collagen binding assay)] method…[is] capable of detecting patients with subtypes 2A and 2B von Willebrand's disease" (Deckmyn I, para. 7). Accordingly, using the collagen binding assay of Favaloro alone cannot differentiate between Type 2A and Type 2B vWD.

Further, given that Hoylaerts' "assay" cannot discriminate between Type 2A and Type 2B vWD for all the reasons discussed above in Sections 3(B)(i-iv), it would not be possible to use Hoyalert's "assay" in addition to Favaloro's collagen binding assay to differentiate between Type 2A and Type 2B, as suggested by the Office action at page 9-10, because neither assay can differentiate between Type 2A and Type 2B vWD.

vi. Conclusion

Because it is not be possible to distinguish between Type 2A vWD and Type 2B vWD using Hoylaert's method for all the reasons discussed above in Sections 3(B)(i-v), Applicants submit that the Office action's asserted motivation for combining the teachings of Favaloro, Vischer, Hoylaerts, and Handin, namely to differentiate Type 2A from Type 2B vWD is flawed. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

C. There is no motivation to use the soluble GP1b(α) fragment of Handin in place of isolated GP1b in the method of Hoylaerts.

The Office action suggests that Handin teaches soluble GP1b(α) (Office action, pg. 11, lines 12-15). The Office action suggests that it would have been obvious to one of ordinary skill in the art to use Handin's soluble GP1b(α) in Hoylaert's assay in place of Hoylaert's GP1b protein because GP1b contains components that are allegedly not involved in the specific binding activity of vWF, while Handin's GP1b(α) allegedly contains the ristocetin-dependent vWF binding site.

Applicants submit that a skilled artisan would have no motivation to use Handin's GP1b(α) fragment in Hoylaert's assay to arrive at Applicants' claimed method of discriminating between different types of vWD because Handin does not teach measuring the binding activity of

vWF in a sample to a soluble form of GP1b(α) that is not associated with a platelet. Rather, Handin teaches that soluble GP1b(α) fragments such as glycocalicin or rGP1b(α)$_{Q221\text{-}L318}$ inhibit ristocetin-dependent binding of vWF to *platelets* and teaches an assay to demonstrate such inhibition ability of glycocalicin or rGP1b(α)$_{Q221\text{-}L318}$. For example, as set forth in column 15, line 52, to column 16, line 3, Handin teaches: "The ability of recombinant GP1b(α) (rGP1b(α)) to inhibit ristocetin-dependent binding of [^{125}I]-vWF to platelets was assessed with *paraformaldehyde-fixed platelets*. …The ability of purified [glycocalicin] or the rGP1b(α) polypeptides to block vWF binding was assessed by adding increasing concentrations of the appropriate test substance to the assay mixture" (emphasis added).

Therefore, Handin teaches a platelet aggregation assay that uses a soluble fragment of GP1b(α) such as glycocalicin or rGP1b(α)$_{Q221\text{-}L318}$ to measure its ability to <u>inhibit</u> vWF binding to *platelets*, thereby reducing platelet aggregation, not to measure the binding activity of vWF in a sample to the soluble fragment of GP1b(α) that is not associated with a platelet. In fact, Handin does not teach any assay to detect the binding activity of vWF in a sample to a soluble fragment of GP1b(α). Moreover, Handin is silent with respect to the application of a soluble fragment of GP1b(α) to detect vWD.

Applicants further submit that a skilled artisan would have no motivation to use Handin's GP1b(α) fragment in Hoylaert's assay to arrive at Applicants' claimed method of discriminating between different types of vWD because Handin does not discuss von Willebrand's disease. Handin's interest in GP1b(α) lies solely in its potential as an antithrombotic treatment to block platelet adhesion (col. 1, lines 17-21 and col. 3, lines 5-6). Further, Handin fails to teach or suggest that GP1b(α) could be useful in a method for differentiating between different types of vWD. In fact, Handin provides no indication that the binding activity between vWF and Handin's GP1b(α) fragments in the absence of platelets would provide data sufficient for making a diagnosis of vWD. For example, Handin, like Hoylaerts, assays only normal vWF and does not assay any defective vWF from vWD patients. Given these deficiencies of Handin, Applicants respectfully submit that a skilled artisan would have no reason to employ the GP1b(α) fragment of Handin in place of the isolated GP1b of Hoylaerts.

Further, Handin, like Hoylaerts, provides no teaching or suggestion that GP1b(α), absent a platelet, is sufficiently robust to bind defective vWF from vWD patients. Given the teachings of Christophe, discussed previously in Section 3(B)(ii), which suggest that soluble GP1b(α) is not sufficiently robust to provide clinically relevant test data to allow accurate discrimination between normal samples and samples from patients with vWD, Applicants submit that a skilled artisan would have no motivation to use the GP1b(α) fragment of Handin in place of isolated GP1b in the method of Hoylaerts.

Further, Applicants' submit that a skilled artisan would have no reasonable expectation of success in using any of Handin's soluble fragments of GP1b(α) in the assay of Hoylaert's to accurately detect the binding activity of vWF in a sample to a soluble form of GP1b(α). For example, Handin teaches that "[n]one of the recombinant polypeptides analyzed…contained the serine threonine-rich region to which O-linked oligosaccharides are attached" (col. 18, lines 45-47). In other words, Handin's recombinant GP1b(α) fragments were devoid of O-linked oligosaccharides. Because glycosylation patterns can be involved in protein-protein binding, Applicants submit that the absence of O-linked oligosaccharides from Handin's recombinant GP1b(α) fragments would preclude a skilled artisan from having a reasonable expectation of success at using those fragments to obtain appropriate levels of binding to vWF necessary for a diagnosis of vWD.

Even though Handin teaches that the biologic activity of rGpIbαL318, one recombinant GP1b(α) fragment, had the same biological activity as glycocalicin, which is a larger, glycosylated naturally occurring proteolytic fragment of GP1b found in circulating plasma, Handin measured the biological activity of the fragment by inhibition of ristocetin-dependent binding of vWF to *platelets*, not by determining the binding activity of vWF to the soluble GP1b(α) fragment (col. 18, lines 48-52). Accordingly, Applicants' submit that Handin's observation regarding the biologic activity of rGpIbαL318 is irrelevant to the effects of those glycosylation patterns on the binding activity of vWF to soluble GP1b(α) as useful for an assay for detecting vWD.

Accordingly, Applicants submit that even if a skilled artisan had a motivation to use a soluble fragment of GP1b(α) of Handin in the assay of Hoylaerts, a skilled artisan would not

have a reasonable expectation that using any of the recombinant GP1b(α) fragments of Handin would produce the requisite biological activity to provide an accurate measurement of vWF binding activity in the sample necessary for making a correct diagnosis of vWD.

For all these reasons, Applicants submit that the teachings of Handin fail to remedy the deficiencies of Hoylaerts, Favaloro, and Vischer. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

D. There is no motivation to avoid non-specific ristocetin-dependent molecular interactions between GP1b and the platelet.

The Office action suggests that a skilled artisan would have been motivated to combine the teachings of Favaloro, Hoylaerts, and Handin and had a reasonable expectation of success in the combination as suggested above because the assay of Hoylaerts has the advantage of detecting vWF without the contributions of non-specific ristocetin-dependent molecular interactions when isolated GP1b is used (Office action, page 6, line 21-page 7, line 1; page 10, lines 6-10).

Applicants respectfully submit that a skilled artisan would not be motivated as suggested by the Office action. In particular, while Hoylaerts states that "in order to avoid non-specific ristocetin-dependent molecular interactions, they did not use whole platelets" (page 462, LH col., discussion, 3rd para.), Hoylaerts teaches that ultimately, avoidance of non-specific ristocetin-dependent molecular interactions depends on the concentration of ristocetin (page 462, LH col., discussion, 3rd para.). For example, Hoylaerts teaches:

> "At increasing concentrations of ristocetin, non-specific vWF binding progressively predominated…The non-specific character of [vWF binding] at ristocetin concentrations exceeding 0.4 mg/ml was confirmed during incubations in plates not coated with GP1b. Therefore, in order to avoid non-specific ristocetin-mediated vWF binding to coated proteins [GP1b], the experimental ristocetin concentrations were limited to 0.4 mg/ml, ristocetin dependent vWF binding to GP1b being specific below this concentration…" (page 457, RH col., 2nd full para.).

Accordingly, based on the teachings of Hoylaerts, while Hoylaerts predicted that the absence of a platelet would avoid non-specific ristocetin-dependent molecular interactions, the data suggest that it was not the absence of the platelet, but rather the concentration of ristocetin that affects the presence or absence of non-specific ristocetin molecular interactions. Accordingly, because Hoylaerts teaches that the ristocetin concentration is the controlling factor, Applicants submit that a skilled artisan would not be motivated to use a GP1b fragment in the absence of a platelet.

Further, given all the reasons discussed above in Section 3B(i) and (ii) with respect to Hoylaerts and Christophe, namely that Hoylaerts failed to test defective vWF binding with isolated GP1b and that Christophe teaches away from using GP1b(α) to detect binding activities of defective vWF in the presence of ristocetin, Applicants submit that taking the teachings of Hoylaerts as a whole, a skilled artisan would have no motivation to combine the teachings of Hoylaerts with Handin, Favaloro, and Vischer to arrive at Applicants' claimed invention. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

E. The rationale of In re Kerkhoven does not obviate the invention because the collagen binding activity and vWF ristocetin cofactor activity are different activities and not useful for the same purpose.

The Office action further supports the combination of Favaloro, Vischer, Hoylaerts, and Handin stating that "it has long been held that it is obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose" (Office action, pg. 11, citing *In re Kerkhoven*, 626 F.2d 846 at 850 (CCPA 1980)). Applicants submit that the Office action's application of this case to the facts is incorrect.

Applicants submit that *In re Kerkhoven* stands for the proposition that "[i]t is *prima facie* obvious to combine two compositions each of which is known in the art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose." *In re Kerkhoven* at 850 (emphasis added). If the Office action is asserting that the assays of Favaloro

(collagen binding assay) and Hoylaerts should be combined to create a third assay, such a combination is not supported by the rationale of *In re Kerkhoven.*

Applicants submit that the rationale of *In re Kerkhoven* does not apply to Applicants' claimed invention because Applicants' invention is a method, not a composition. Likewise, the collagen binding assay, is a method, not a composition.

However, even if the rationale of *In re Kerkhoven* applies to method claims, which Applicants submit that it does not, as stated in *In re Kerkhoven*, the combination of elements must be used for the very same purpose. Applicants submit that neither the assay of Favaloro or Hoylaerts is used for the very same purpose. As asserted by the Office action, Favaloro teaches a collagen binding assay (Office action, pgs. 9, lines 2-3). The collagen binding assay for detecting vWF activity "primarily involves the functional domain A3 of mature vWF" (Deckmyn I, para. 7b). In contrast, binding of vWF to the GP1b complex involves the functional domain A1 of vWF (Deckmyn I, para. 7b). Accordingly, the purposes of the collagen binding assay and an assay detecting vWF/GP1b binding are different, namely to detect defects in differing functional domains of vWF. In fact, Dr. Deckmyn indicates that whether the collagen binding assay or the ristocetin cofactor assay (which involves binding of vWF to the GP1b) is employed, "materially affects the diagnosis of von Willebrand disease" (Deckmyn I, para. 8).

The Office action has stated at page 17 of the Office action that while the collagen binding assay and the ristocetin cofactor activity assay, the activity detected in step (a) of Applicants' claimed invention, may detect different functional domains of vWF, they have same purpose of determining vWF binding activity. Accordingly, the Office action submits that the rationale for combining the teachings of Favaloro and Hoylaerts is valid. Applicants again respectfully disagree.

The collagen binding activity and the ristocetin cofactor activity are two different binding activities, *i.e.*, they each measure the ability of vWF to bind to different substrates. For example, the collagen binding activity is a measure of vWF bound to immobilized collagen and is used to detect the presence of HMW multimers in a plasma sample (Deckmyn I, para. 7c). In contrast, a ristocetin cofactor assay measures the ability of vWF to bind to GP1b and is sensitive to the total level of vWF in the blood, not just HMW multimers (Deckmyn I, para. 7c). Accordingly, the

collagen binding activity and the ristocetin cofactor activity are determined for different purposes. The collagen binding activity is determined to detect the content of HMW and the ristocetin binding activity to detect total vWF in a patient (Deckmyn I, para. 7c). Applicants submit that these are clearly different purposes.

Further, Dr. Deckmyn states that "the CBA [(collagen binding assay)] and the ristocetin cofactor assay were not art recognized equivalents at the time [Applicants'] invention" (Deckmyn I, para.4). In other words, the collagen binding activity and the ristocetin cofactor activity do not serve equivalent purposes. As such, these assays cannot be combined under the rationale of *In re Kerkhoven* to form a third assay as the Office action suggests because each of the individual assays cannot be used for the very same purpose.

For all these reasons, Applicants submit that the claimed invention is not obvious in view of *In re Kerkhoven*. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

4. The Office action has failed to explain how steps (b) through (e) of Applicants' claimed invention are obvious with respect to detecting vWF binding activity to GP1b(α).

The Office action asserts at page 4 that Favaloro teaches steps (a) through (e) of Applicants claimed method except that Favaloro teaches a collagen binding assay, not the binding of vWF to a soluble form or portion of glycoprotein 1bα in the presence of ristocetin (page 5, lines 14-16). The Office action suggests that the collagen binding assay of Favaloro be employed with Hoylaerts' method (page 9, line 19-page 10, line 5). The Office action asserts that Hoylaerts teaches detecting vWF binding activity to GP1b in the presence of ristocetin (page 6, lines 8-10). Accordingly, even if a skilled artisan performed the collagen binding assay as taught by Favaloro in addition to the method taught by Hoylaerts, Applicants submit that the Office action has failed to provide a rationale for performing the method of Hoylaerts in conjunction will all remaining steps required by Applicants' claimed invention, *i.e.*, steps (b) through (e) of claim 31.

Applicants' claimed invention requires (a) detecting a binding activity of vWF in a sample as claimed, (b) determining an amount of vWF antigen in that sample, (c) determining a

ratio between the binding activity of vWF in step (a) and the amount of vWF antigen in step (b) is determined for the sample, (d) comparing the ratio to a reference range, and (e) detecting vWD based on the comparison in step (d) as claimed.

However, while arguably the Office action has asserted that the combination of Hoylaerts and Handin teaches step (a), which Applicants submit they do not, Applicants fail to see where the Office action has provided a rationale to perform steps (b) through (e) of the claimed invention as recited in claim 32. Because steps (b) and (c) must be performed on the same sample as step (a) and steps (d) and (e) reference steps (c) and (d) respectively, Applicants submit that the alleged performance of claimed steps (b) through (e) in the collagen binding assay taught by Vischer does not suffice as a teaching for performing steps (b) through (e) with respect to the method of Hoylaerts'. Favaloro only teaches performing steps (b) through (e) with respect to a collagen binding assay.

Accordingly, Applicants submit that the Office action has failed show that each and every step of Applicants' claimed invention is obvious in view of the references cited. Accordingly, Applicants respectfully request that the rejection of claim 31 and claims 32, 33, 36-41, 50-53, 56, 60, and 62 depending therefrom under 35 U.S.C. § 103 be reconsidered and withdrawn.

II. Rejection of Claim 54 under 35 U.S.C. § 103(a) further in view of Batz

Claim 54 stands rejected under 35 U.S.C. § 103(a) further in view of Batz *et al.* (U.S. Patent No. 4,415,700 "Batz"). Applicants submit that Batz fails to remedy the deficiencies of Favaloro, Vischer, Hoylaerts, and Handin, all of which fail to teach or suggest, either alone or in combination, detecting the binding activity of vWF in the presence of ristocetin to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in a method for discriminating between different types of vWD.

Batz teaches hydrophilic latex particles useful as carriers for immunologically active substances (col. 1, lines 5-9). Batz does not teach detecting the binding activity of vWF in the presence of ristocetin to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in a method for discriminating between different types of vWD. Batz does not even teach GP1b(α) or an anti-GP1b(α) antibody. Accordingly, Applicants submit that it would not be obvious, based on the teachings or Batz, to present GP1b(α) via an anti-GP1b(α) antibody in

order to detect vWF binding activity to GP1b(α) in the presence of ristocetin. Accordingly, Applicants submit that claim dependent claim 54 is patentable at least for the same reasons that independent claim 31 from which it depends is patentable. Applicants therefore respectfully request reconsideration and withdrawal of the rejection of claim 54 under 35 U.S.C. § 103 in view of Batz.

III. Rejection of Claim 55 under 35 U.S.C. § 103(a) further in view of Solen

Claim 55 stands rejected under 35 U.S.C. § 103(a) further in view of Solen *et al.* (U.S. Patent No. 6,043,871 "Solen"). Applicants submit that Solen fails to remedy the deficiencies of Favaloro, Vischer, Hoylaerts, and Handin, all of which fail to teach or suggest, either alone or in combination, detecting the binding activity of vWF in the presence of ristocetin to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in a method for discriminating between different types of vWD.

Solen teaches an instrument for measuring platelet aggregation (abstract). Solen does not teach detecting the binding activity of vWF in the presence of ristocetin to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in a method for discriminating between different types of vWD. Solen does not even teach GP1b(α) or a fragment thereof that is not associated with a platelet or an anti-GP1b(α) antibody. Accordingly, Applicants submit that it would not be obvious, based on the teachings or Solen, to present GP1b(α) via an anti-GP1b(α) antibody in order to detect vWF binding activity to GP1b(α) in the presence of ristocetin.

IV. Rejection of Claim 61 under 35 U.S.C. § 103(a) further in view of Vicente

Claim 61 stands rejected under 35 U.S.C. § 103(a) further in view of Vicente (J. Biol. Chem., 263:18473-18479 (1988) ("Vicente")). Applicants submit that Vicente fails to remedy the deficiencies of Favaloro, Vischer, Hoylaerts, and Handin, all of which fail to teach or suggest, either alone or in combination, detecting the binding activity of vWF in the presence of ristocetin to a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in a method for discriminating between different types of vWD.

Vicente teaches a fragment of GP1b(α) (glycocalicin) that is capable of interacting with purified surface-bound vWF (*see*, *e.g.*, Vicente, abstract, pg. 18475, LH col.). Vicente also teaches an anti-GP1b antibody used in an antibody inhibition assay to impair glycocalicin binding to vWF (p. 18465, FIG. 6 legend).

In contrast to the claimed invention in which an antibody is used to present GP1b(α) to detect the binding activity of vWF, Vicente does not use an anti-GP1b(α) antibody to present GP1b(α), as required by Applicants' claimed invention. Rather, Vicente reports that anti-GP1b antibodies "inhibited [glycocalicin's] interaction with vWF to a variable extent" (p. 18465, FIG. 6 legend). Given Vicente's teaching that anti-GP1b antibodies inhibit glycocalicin's ability to bind vWF, a skilled artisan would not be motivated to use an anti-GP1b(α) antibody to present GP1b(α) for binding to vWF given that it could interfere with vWF binding to GP1b(α), the binding activity determined according to Applicants' claimed invention.

In addition, Vicente, like Hoylaerts and Handin does not assay patient samples to detect vWF binding activity to GP1b(α), as required by Applicants' claimed invention. Rather, Vicente's vWF is fixed to plates and glycocalicin is added. Accordingly, Vicente provides no indication that glycocalicin would bind to non-immobilized vWF from a patient sample or that glycocalicin would be robust enough to bind to defective vWF found in a patient sample.

Further, Vicente, like Hoylaerts and Handin, does not assay any defective vWF from vWD patients. Accordingly, Vicente provides no suggestion that glycocalicin can bind sufficiently with defective vWF, let alone that binding activities detected between glycocalicin and defective vWF would be sufficient to provide clinically relevant results for discriminating between types of vWD as required by Applicants' claimed invention.

For these reasons, Applicants submit that Vicente fails to remedy the deficiencies of Favaloro, Vischer, Hoylaerts, and Handin as applied to claim 31. Accordingly, Applicants submit that claim 61 is patentable at least for the same reasons that claim 31 is patentable and respectfully request reconsideration and withdrawal of the rejection of claim 61 under 35 U.S.C. § 103.

V. Applicants invention is not obvious because it meets a long-felt need in the art for an improved assay for detecting the binding activity between vWF and GP1b, i.e., ristocetin cofactor activity.

Applicants submit that Applicants' invention is not obvious because Applicants' invention satisfies a long felt need in the art for an improved ristocetin cofactor activity assay for diagnosing vWD.

Applicants submit that the requirements for establishing non-obviousness based on long-felt need (See MPEP § 716.04 I) are met. In particular, Applicants submit that the long-felt need for an improved ristocetin cofactor activity assay i) has been persistent and recognized by those of ordinary skill in the art, ii) was not satisfied by others before Applicants' invention, and iii) is satisfied by Applicants' claimed invention for the reasons set forth below.

1. The long-felt need has been recognized by others skilled in the art and has persisted since those skilled in the art recognized the need.

Applicants invented the first assay capable of detecting vWF ristocetin cofactor activity using GP1b(α) presented by an anti-GP1b(α) antibody. Prior to Applicants' invention, detecting the ristocetin cofactor activity of vWF in a sample required using GP1b associated with a platelet. Since the ristocetin cofactor activity assay was first developed in the early 1970s, those skilled in the art of diagnosing vWD have recognized several deficiencies in the accuracy, sensitivity, and reproducibility of the prior art assays (see Bruguera I, paras. 6-7).

In particular, as stated by Dr. Bruguera in his first declaration ("Bruguera I"):

> "[t]here has been a long-felt need in the field of von Willebrand testing and diagnosis since the time the ristocetin cofactor activity assay was first developed for an improved ristocetin cofactor activity assay that significantly reduces or eliminates intra and interassay variability and improves sensitivity for measuring von Willebrand factor, *i.e.*, an assay that can measure the low levels of von Willebrand factor characteristic of severe von Willebrand disease subtypes with levels of von Willebrand factor below 20%" (Bruguera I, para. 8).

Even as recently as 2007, Favaloro (Favaloro, (2007), *Seminars in Thrombosis and Hemostasis*, 33(8):727-744, "Favaloro II," attached as "Exhibit B" to Bruguera I and Bruguera II) acknowledged that "over the subsequent 35 years or so [since the assay was first introduced],

several significant limitations to the ristocetin cofactor assay have emerged" (Favaloro II, pg. 729, RH col.). In particular, as outlined in Favaloro II and described by Dr. Bruguera in his first declaration filed August 20, 2008, ("Bruguera I") at paragraphs 6-7 and in his second declaration at paragraphs 5-8 (Second Declaration of Dr. Pablo Bruguera filed herewith, "Bruguera II"), the prior art ristocetin cofactor activity assay has suffered from poor interassay variability, poor intra-assay variability, poor reproducibility, and low sensitivity. Poor intra-assay and interassay variability refers to high coefficients of variation (%CV), while low sensitivity indicates that these prior art assays are not sensitive enough to accurately and reliably detect low levels of vWF activity (%vWF) (Bruguera II, paras. 5 and 6). As a result, prior art ristocetin cofactor activity assay "cannot reliably provide an estimate of von Willebrand factor below around 20%...[which] is a serious limitation" (Favaloro II, pg. 730, LH col.). Given that many vWD patients have vWF activities lower than 20%, 10%, 5%, and even 1%, the inability of the prior art ristocetin cofactor activity assay to reliably detect extremely low levels of vWF activity makes it extremely difficult to accurately diagnose these patients (Bruguera II, para. 6). This is confirmed by Favaloro who teaches that the limitations of the prior art ristocetin cofactor assay described above create a "high potential error rate in terms of false positive and false negative identification of vWD" (Favaloro II, p. 730, RH col., first para.).

The known limitations of the prior art ristocetin cofactor activity assay in diagnosing vWD have been acknowledged by others in the art, not just Favaloro and Dr. Bruguera. For example, the National Institutes of Health December 2007 Report entitled "The Diagnosis, Evaluation, and Management of von Willebrand Disease" ("NIH Report," attached as "Exhibit B") states that:

> "The ristocetin cofactor activity (VWF:RCo) assay has high intra and interlaboratory variation…the coefficient of variation (CV) has been measured in laboratory surveys at 30 percent or greater…This becomes important not only for the initial diagnosis of VWD, but also for determining whether the patient has type 1 versus type 2 vWD" (NIH Report, page 27, LH col.).

Further, the NIH Report confirms Favaloro's assertion that the prior art ristocetin cofactor activity assays lack sensitivity to low levels of vWF and cannot accurately detect below 10-20% vWF. In particular, the NIH report asserts that "the CV is still higher [than 30%] when

the vWF is lower than 12-15 IU/dL [(12-15% vWF)]" (NIH Report, page 27, LH col.). In other words, for vWF below 15%, the coefficient of variation is greater than 30%. Having a coefficient of variation greater than 30% indicates that these assays are not precise enough to give accurate measurements at these lower levels of vWF, as the generally accepted industry standard for variation in these types of assays less than 15% (Bruguera II, para. 9). Such an error level would significantly and negatively effect the accuracy of a diagnosis of vWD (Bruguera II, para. 9).

Applicants submit that the need for an improved ristocetin cofactor activity assay has persisted since the deficiencies with the assay were first recognized in the early 1970s (Bruguera II, para. 7). For example, that Favaloro and the NIH Report were complaining about the deficiencies of the prior art ristocetin cofactor activity assay as recently as 2007 indicates that the assay's problems still persist. Further, according to Dr. Bruguera, until the advent of Applicants' claimed invention, the known problems with the prior art ristocetin cofactor assay had not been satisfactorily addressed (Bruguera II, para. 10).

For all these reasons, Applicants submit that since the 1970s, there has been a recognized and persistent need in the art for an improved ristocetin co-factor activity assay having the sensitivity to detect low levels of vWF and with reduced levels of intra and interassay variability.

2. Others had not satisfied this long felt-need prior to the invention by Applicants.

Applicants submit that others had not satisfied the need for an improved ristocetin cofactor activity assay as of the priority date of this application.

Others have made attempts to improve the prior art ristocetin cofactor assay in the years since the deficiencies of the assay were first elucidated (Bruguera II, para. 10). For example, laboratories have used stabilized platelets obtained from suppliers rather than preparing their own platelets in order to reduce the variability caused by each laboratory using platelets from different sources (Bruguera II, para. 10). Further, laboratories have automated the prior art ristocetin cofactor assay to reduce error in detecting agglutination of platelets, to reduce the time-intense nature of the assay, and to computerize the complex series of calculations involved in measuring platelet agglutination to reduce human error (Bruguera II, para. 10). However, none of these efforts to improve the prior art ristocetin cofactor activity assay has been successful in

providing the needed improvements in inter- and intra-assay variability and sensitivity (Bruguera II, para. 10).

For example, an exemplary commercially available ristocetin cofactor activity assay, available as early as January 1999 (Bruguera I, paras. 9-16 and "Exhibit C"), and discussed by Dr. Bruguera in his first declaration has not solved the problems of the prior art ristocetin cofactor activity with respect to inter-assay and intra-assay variability and sensitivity. While the exemplary commercially available assay may have improved inter-assay and intra-assay variability due to performance of the assay in an automated system, "even automated assays…have not fully addressed all the drawbacks of the classical ristocetin cofactor assay" (Bruguera I, para. 10). For example, as stated by Favaloro, "automation of test procedures (using instrumentation) has certainly reduced the ristocetin cofactor activity assay's intra-assay and inter-assay variability, ***but has not alleviated the issue of low-level assay sensitivity***, nor does automation seem to protect laboratories against vWD identification errors" (Favaloro II, pg. 730, LH col., emphasis added).

For example, as indicated by Favaloro, an assay with the ability to accurately detect vWF activity below 20% is needed (Favaloro II at 730, LH col.). The product specifications for the commercially available assay discussed by Dr. Bruguera provides no indication that it can accurately detect levels less than 20% vWF; in fact, the product specification does not indicate the lower limit of quantitation for the assay (Bruguera II, para. 12). In addition, the coefficients of variation for the commercially available assay's pathological control, *i.e.*, a sample with a known low level of vWF, ranges as high as 16.2% (within run) 16.9% (total) (Bruguera II at para. 12). It would not be possible to accurately and precisely quantitate low levels of vWF, *i.e.*, below 10-20% vWF, because the coefficients of variation for this assay exceed the generally accepted industry standard for precision which is less than 15% CV (Bruguera II at para. 12). Accordingly, "further improvements in inter-assay and intra-assay variability were still necessary as of the priority date" of this application (Bruguera I, para. 10).

In addition, despite the known deficiencies in the prior art ristocetin cofactor activity assay's ability to provide reliable detection of vWF activity, the NIH Report indicated in 2008 that the prior art ristocetin cofactor assay is still "the most widely accepted laboratory measure of

vWF function" (NIH report, page 27, sentence bridging LH and RH col.). That these assays are still being used despite their deficiencies is further evidence that others have not satisfied the need for an improved assay, as there is no satisfactory alternative. Moreover, that Favaloro and the NIH Report have both reported the deficiencies of the prior art ristocetin cofactor activity assays as recently as 2007 is further evidence that the prior art ristocetin cofactor assay has not yet been satisfactorily improved by others.

For all these reasons, Applicants submit that others have not satisfied the long felt need for a ristocetin cofactor activity assay with the necessary improved sensitivity to accurately detect low levels of vWF and to eliminate the very high coefficients of variation typically seen with the prior art ristocetin cofactor assay.

3. Applicants' invention satisfies the long felt need of others skilled in the art.

While Applicants' claimed assay is not yet available commercially, those of skill in the art who are aware of Applicants' assay acknowledge that it fulfills the long felt need of skilled artisans in the field of vWD diagnosis for a more precise and accurate assay for detecting ristocetin cofactor activity. For example, Dr. Bruguera has indicated that until the advent of Applicants' claimed invention, the known problems with the prior art ristocetin cofactor assay had not been satisfactorily addressed (Bruguera II, para. 10).

Applicants assay has satisfied this long felt need by providing further reductions in the coefficients of variation as compared to prior art ristocetin cofactor activity assays, and by providing the requisite low level sensitivity to detect levels of vWF below 20%, 10% and 1% and even 0.5% vWF (Bruguera II, para. 16).

For example, in contrast to the prior art ristocetin cofactor assays which "cannot reliably provide an estimate of vWF below around 20%" (Favaloro II, p.730, LH col., 1st para.), Applicants' claimed assay can provide detection levels at least as low as 1% vWF, overcoming the "serious limitation" of the prior art assay acknowledged by Favaloro (Favaloro II, pg. 730, LH col., 1st para.). In fact, Applicants' assay has a lower limit of quantitation of 0.27% vWF with a coefficient of variation of 7.9%, well below the generally accepted industry stand of 15% CV (Bruguera II, paras. 8 and 11). Further, Applicants' lower limit is 30-60 times lower than the 10-20% lower limit of the prior art ristocetin cofactor activity assay as reported by Favaloro

(Favaloro II, p.730, LH col., 1st para.). As stated by Dr. Bruguera, this is the lowest known lower detection limit for any ristocetin cofactor activity assay available as of the priority date of this application (Bruguera II, para. 11). Accordingly, Applicants' assay meets the long felt need in the art for a ristocetin cofactor activity assay that can detect <10-20% vWF as Applicants assay is 30-60 times more sensitive than the lower detection limit of 10-20% for prior art ristocetin cofactor assays as reported by Favaloro (Favaloro II, pg 730, LH col. 1st para.)

Further, Applicants assay provides reductions in coefficients of variation beyond those of the prior art ristocetin cofactor activity assays. Coefficients of variation for Applicants' assay are shown in Table 1 of Dr. Bruguera's second declaration (Bruguera II, Table 1). As stated by Favaloro, coefficients of variation for prior art ristocetin cofactor activity assays are reported in the range of 20-40% (Favaloro II, LH col., 3rd para.). These lower coefficient of variation obtained from Applicants' claimed assay, in comparison to the coefficient of variation for the prior art ristocetin cofactor activity assays, including the exemplary commercially available ristocetin cofactor activity assay discussed above, demonstrate a significant and valuable improvement in reducing the inter- and intra-assay variability of the ristocetin cofactor activity assay. Applicants improvements in reducing total and within run variation indicates that the mean % vWF activity detected in patient samples according to Applicants' claimed method with be more accurate, and as a result, a more accurate diagnosis of vWD can be made (Bruguera II, para. 14). Accordingly, for these reasons Applicants have met the long-felt need in the art for a ristocetin cofactor activity with reduced levels of variability.

Further evidence that Applicants' assay has met the long-felt need is found in the NIH Report which refers to ELISA assays that assess direct binding of a person's plasma vWF to GP1b derived from plasma glycocalicin (pg. 27, LH col.). In support of this assay, the NIH Report references a paper published in 2000, after the priority date of this application, which is authored by the inventors of this application and which generally describes the claimed invention (Deckmyn *et al.*, (2000), "A reliable and reproducible ELISA method to measure ristocetin cofactor activity of von Willebrand Factor," Thromb. Haemost., 83:107-113, attached as Exhibit C). The NIH Report acknowledges that Deckmyn's method, which is the subject of the instant application, can detect below 1U/dL of vWF (*i.e.*, below 1% vWF), see Deckmyn 2000 at 112,

LH col.). The other assays reported by the NIH, which require use of platelet-GP1b, have sensitivity limits of 6-12 IU/dL and 10-20 IU/dL, but with coefficients of variation greater than 30% in these ranges, especially when measuring below 12-15 IU/dL (NIH report, pg. 27, LH col.). As previously discussed, these high coefficients of variation mean that these prior art assays lack the necessary sensitivity to precisely and accurately detect below 10-20% vWF. This contrasts with Applicants' assay, which has coefficients of variation ranging from 3.5%-7.2% (Bruguera II, Table 1). That the NIH report has acknowledged the increased sensitivity of Applicants' claimed invention over the other assays is evidence that Applicants' assays has met the long-felt need in the art.

Applicants submit that as of the priority date of this Application, Applicants' claimed invention was the only known ristocetin cofactor activity assay with the sensitivity to accurately detect less than 1% vWF with acceptable levels of inter-assay and intra-assay variation (CV%) (Bruguera II, para. 16). Further, because Applicants' claimed assay has the ability to reliably detect levels of vWF well below 1%, *i.e.*, as low as 0.27%, and because it significantly reduces levels of intra-assay and inter-assay variability, the long felt need in the art for an improved ristocetin cofactor activity assay with the requisite sensitivity to precisely and accurately detect low levels of vWF with satisfactorily low coefficients of variation has been met by Applicants' claimed invention.

Further, Applicants submit that the improvements in inter-assay and intra-assay variability as well as sensitivity achieved by Applicants' assay result from the limitations of the claimed invention. As stated by Dr. Bruguera, the success of Applicants' assay "can be attributable to the use of a soluble form or portion of the glycoprotein 1b(α) presented by an anti-GP1b(α) antibody" (Bruguera II, para. 16).

Applicants further submit that the patentability of Applicants' claimed invention is evidenced by the fact that in all the years intervening since the development of the ristocetin cofactor assay, no one has attempted to improve the ristocetin cofactor assay by using a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet, let alone an assay where the soluble form or portion of GP1b(α) is presented by an anti-GP1b(α) antibody. If such

an improvement were obvious, Applicants respectfully question why no one else had done so prior to Applicants' earliest effective filing date.

4. Conclusion

For all the reasons set forth above in Sections V(1), (2), and (3), Applicants submit that their claimed invention is not obvious because it meets a long felt need in the art for an improved ristocetin cofactor activity assay capable of accurately and precisely detecting extremely low levels of vWF (*i.e.*, less than 10-20%) with acceptable levels of inter-assay and intra-assay variation (CV%), a need not previously met by others, a need that has been recognized since the early 1970s. Accordingly, Applicants respectfully request that the rejection of the pending claims under 35 U.S.C. 103(a) be reconsidered and withdrawn.

V. Applicants invention is not obvious because it achieves unexpected results.

Applicants submit that Applicants' claimed invention is also not obvious in view of the unexpected results that Applicants' assay demonstrates over the prior art. In particular, Applicants' assay requires the step of detecting a binding activity of vWF in a sample to a soluble form or a portion of GP1b(α) that is presented by an anti-GP1b(α) antibody in the presence of ristocetin or a functionally equivalent substance. This step is a ristocetin cofactor activity assay (see Bruguera I, paras. 4-5).

The classical ristocetin cofactor activity assay has been available since the early 1970s (Favaloro II, pg. 720, RH col.). However, as discussed above, the classical ristocetin cofactor activity assay has suffered from poor intraassay reproducibility, high interassay variability, and high interlaboratory variability. Further, the classical ristocetin cofactor activity assay has proven unreliable at estimating vWF below around 20% (100% being normal) (Favaloro II, pg. 730, LH col., lines 3-14).

Given the poor reproducibility, high variability, and proven unreliability of the prior art ristocetin cofactor activity assay at low levels of vWF in a patient sample, the skilled artisan would predict that a modified ristocetin cofactor activity assay would exhibit similar drawbacks. Surprisingly and unexpectedly, Applicants' assay demonstrates significant improvements in

reproducibility, variability, and reliability in detecting low levels of vWF as compared to the prior art ristocetin cofactor activity assay for the reasons provided below.

1. The prior art suggested that GP1b(α) would not be sufficiently robust to distinguish between normal vWF and vWF from vWD patients.

Firstly, Applicants submit that the fact that the claimed assay is sensitive enough to be able to detect vWF activity in normal and vWD patients, even patients having as little vWF activity as 0.27%, is unexpected in view of the prior art teachings. As previously stated, Applicants' claimed assay requires the step of detecting a binding activity of vWF in a sample to a soluble form or a portion of GP1b(α) that is presented by an anti-GP1b(α) antibody in the presence of ristocetin or a functionally equivalent substance. However, the prior art suggests that GP1bα is not sensitive or robust enough to detect binding activity of *defective* vWF and normal vWF is supported by the teachings of Christophe ((1994),Blood, 83(12):3553-3561), cited in the October 23, 2006, Office action. Christophe compares the binding capacity of *defective* vWF from type 2A vWF, type 2B vWF, and normal vWF to a soluble fragment of GP1b(α), glycocalicin (pg. 3554, LH col., lines 1-4). Christophe found that the binding of plasma vWF from type 2A vWD patients, type 2B vWD patients, and normal patients to glycocalicin in the presence of saturating concentrations of botrocetin, a functional equivalent of ristocetin, "*was not significantly different*" (Christophe, pg. 3560, LH col. 2nd paragraph, abstract 2nd col., emphasis added). In other words, Christophe disclosed that the binding activity detected between normal and defective vWF in a plasma sample to a soluble fragment of GP1b(α), glycocalicin, did not provide clinically relevant data to allow discrimination between samples from normal patients and samples from patients with vWD.

Accordingly, based on the teachings of Christophe, it would not be expected that GP1b(α) would be useful in an assay to detect the vWF binding activity in the presence of ristocetin in order to discriminate between different types of vWD because the binding of GP1b(α) to vWF wouldn't have been expected to be different between normal and vWD patients.

In the face of teachings of Christophe which would lead the skilled artisan away from using GP1b(α), Applicants' discovered that GP1b(α), when presented by an anti-GP1b(α) antibody is sufficiently sensitive enough to detect vWF activity in vWD patients. Surprisingly in

view of the prior art, Applicants' claimed assay detects levels of vWF as low as 0.27% vWF, indicating that GP1b(α) is so unexpectedly sensitive that levels of vWF in vWD patients that are less than 1% may be detected (Bruguera II, para. 6).

For these reasons, Applicants submit that it was unexpected that a soluble form or portion of GP1b(α) could be successfully used to detect the binding to vWF in the presence of ristocetin in a method to discriminate between different types of vWD.

Further, that Applicants' claimed assay has the ability to detect vWF binding activity to GP1b(α) in the presence of ristocetin as low as 0.27% vWF with coefficients of variation well below the generally accepted industry standards for precision (*i.e.*, less than 15%CV) is unexpected.

As discussed previously, Favaloro teaches that the prior art ristocetin cofactor activity assays have a "serious limitation" in that they cannot reliably provide an estimate of vWF below around 20% vWF" (Favaloro II, p. 730, LH col., 1st para.). However, Applicants' ability to detect vWF levels as low as 0.27% vWF indicates that Applicants' claimed assay is 30-60 times more sensitive than the prior art ristocetin cofactor assays' "lower limit of detection which typically lies around 10 to 20% vWF" (Favaloro II, p. 730, LH col., 1st para.).

Applicants submit that even if it were obvious to use a soluble form or portion of GP1b(α) presented by an anti-GP1b(α) antibody in order to detect the ristocetin cofactor activity of vWF instead of using a whole platelet, which Applicants submit it is not, Applicants submit that it could not be expected to achieve a 30-60 fold increase in sensitivity to vWF with satisfactory levels of variation, especially in view of the teachings of Christophe, discussed above, which suggest that GP1b(α) would not be sensitive enough to discriminated between the vWF from normal patients and the vWF from vWD patients.

2. Conclusion

For all these reasons, Applicants submit that Applicants' invention is not obvious because the results obtained from the claimed invention are unexpected. Accordingly, Applicants respectfully request that the rejection of the pending claims under 35 U.S.C. 103(a) be reconsidered and withdrawn.

CONCLUSION

Applicants believe that the pending claims are now in condition for allowance. The Examiner is invited to telephone the undersigned attorney to discuss any remaining issues. Early and favorable actions are respectfully solicited.

Respectfully submitted,

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